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IMPROVING THE QUALITY PROFILE OF DRY BEANS FOR THE CANADIAN PRAIRIES USING GENETIC AND METABOLOMIC APPROACHES

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IMPROVING THE QUALITY PROFILE OF DRY BEANS FOR THE CANADIAN PRAIRIES USING GENETIC AND METABOLOMIC APPROACHES

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1. Abstract

This project was designed to investigate seed coat quality issues in dry bean. The genetic and biochemical basis for differences in post-harvest darkening in pinto beans was determined. The effect of genetics and environment on pre-harvest yellowing of some pinto bean genotypes was established. Cooking and canning quality parameters were assessed for shiny vs matte black beans and no real difference was observed in the first year of trials. Knowledge gained form this project will lead to the development of a science-based marketing strategy for Saskatchewan-grown dry beans

2. Executive Summary

Seed coat quality is of utmost importance when selling dry beans as it is the primary determinant of price after market class. This project looked at three issues related to seed coat quality in bean: post-harvest seed coat darkening in pinto and related classes; pre-harvest yellowing of pinto bean seed coats; and the processing quality of shiny vs matte beans.

Post-harvest seed coat darkening is a significant problem in pinto beans, resulting in product that is undesirable to consumers and that is discounted in the marketplace. There is a range in the rate and extent of darkening among pinto germplasm and recently, slow-darkening lines have been identified. To incorporate the slow-darkening trait into new cultivars there is a need for a quick, reliable, and inexpensive method to accelerate darkening without affecting seed germination. Such a test was developed based on UVC light. It is quick, consistent over years, economical and has no effect on seed germination. A genotype by environment study was conducted to validate this protocol. After accelerated darkening, line and environment effects were found to be significant ($p<0.0001$) but the genotype by environment interaction was not significant ($p=0.29$), which indicated that the UVC protocol could be used to distinguish slow-darkening pinto beans from darkening pinto beans, regardless of where the beans were grown.

To determine the genetic control of the slow darkening trait, line 1533-15, a slow darkening line from the University of Saskatchewan, was crossed to CDC Pintium and HR99, regular darkening pintos, and seed of F_1 and F_2 individuals and $F_{5:6}$ recombinant inbred lines (RILs) were assessed for their darkening phenotype. Results from all populations examined demonstrated that a single gene governs the slow vs regular darkening status of an individual but that there are likely other genes involved in the extent to which a given line darkens. The simple genetics of this trait has facilitated the introduction of this trait into the pinto and carioca breeding programs, thereby increasing the visual quality of beans being developed. A visual marker was identified that will aid in selection: seedlings of slow darkening individuals have greener stems than do regular darkening individuals.

Metabolic analyses of the overall level of proanthocyanidins using a vanillin assay demonstrated that aged and non-aged seed coats of CDC Pintium had significantly higher levels of proanthocyanidins than aged and non-aged 1533-15 seed coats. Aged and non-aged seed coats of both lines were found to contain one main flavonol monomer, kaempferol. The concentration of kaempferol in seed coats of CDC Pintium was significantly higher than in seed coats of 1533-15, although the amount dropped after aging in CDC Pintium but not in 1533-15. The content of kaempferol decreased nearly by half in the seed coats of CDC Pintium after aging, whereas no significant change was observed in the seed coats of 1533-15. Analysis of a subset of RILs indicated that the reduced level of kaempferol and PPO activity was present in all slow-darkening individuals analyzed.

Pre-harvest yellowing of pinto bean seed coats appears to be related to the environment during harvest. The phenomenon seems to appear only in years with wet harvest conditions and only affects a subset of seeds from a field suggesting it is related to maturity when exposed to the unfavourable conditions. Producers are being reminded of the importance of timely harvests. As the phenomenon seems to be worse in certain pinto bean cultivars, there is probably a genetic component. CDC Pintium has been noted to yellow in some years. There did not appear to be any genetic relationship between individuals grown from yellowed seed and any particular sub-line(s) of the breeder seed. This suggests that it is a general trait of the cultivar and only a few plants or seeds are susceptible at the time the crop is exposed to unfavourable conditions. Breeders would be well advised to take advantage of the availability of seed samples from sites with wet harvest conditions to try to identify and eliminate the worst lines before they become cultivars.

The hypothesis that shiny beans are inferior in quality to matte beans, based on negative comments from the trade and consumers, did not generally appear to hold up in the populations observed under this project. Sister lines that were contrasting for shiny and matte seed coat lustre were grown in three environments and subjected to a canning trial. There was no difference between the groups in cooking time as measured by hydration coefficient. With the exception of one location, there were no differences between the groups in any of the canning quality parameters investigated. At the SPG site, there were minor differences in texture and colour between the two groups. A second year of testing will have to be conducted to confirm the results.

Much of the research conducted as part of this project will help Saskatchewan bean exporters develop a science-based marketing strategy in domestic and overseas markets. Our understanding of genetic control of the seed coat darkening phenomenon for pinto beans will form the basis for establishing improved economic value relative to regular pinto beans, forming the basis of a value chain for marketing in the highest priced sector of the bean market.

3. Technical Report

3.1 Introduction

Quality of the bean crop is based primarily on visual characteristics of seeds and seed coats with discoloration, staining and colour retention during processing as some of the major considerations taken into account by buyers and exporters. Little is known of the cause or the control of these phenomena. We are investigating these aspects of quality with the intent of

providing breeders and producers with the tools to develop and produce higher quality beans for western Canada.

The overall project had three major objectives:

1) To identify the cause of seed coat discoloration in pinto bean and transfer the gene(s) responsible for the slow-darkening phenotype to elite pinto, flor de mayo and carioca breeding lines;
2) To identify the cause of yellow staining in pinto bean; and
3) To investigate the relationship between shiny seed coats and cooking and canning quality.

3.1.1. Discoloration of pinto beans with storage.

Seeds of common bean (*Phaseolus vulgaris* L.), like other pulses, are sold based on visual characteristics such as size, shape and colour. Darkening of the seed coat during storage is a significant problem in several market classes, particularly pinto and carioca beans. Merchants and consumers generally assume that beans that have darkened are older and more difficult to cook. This can result in a loss of economic value through discounted pricing. Some varieties darken more quickly than others and, as a result, are downgraded more often than those that retain their bright background colour.

On a Pulse Canada marketing mission to Mexico in April 2002, all pinto bean buyers consistently identified the sample with the brightest background as their preference. They complained of the quality of pintos coming out of the Midwest USA and Manitoba based primarily on darkened seed coats. Pinto lines that appear to have brighter seed coats that darken much more slowly than normal pintos have been identified in breeding lines at the University of Saskatchewan. At the start of this project, the genetic control of this phenomenon was not known although it was assumed to be simply inherited. Selecting for this trait in a timely fashion depends on the development of an accelerated darkening protocol as waiting for the seeds to darken naturally takes months. As the seed coat and therefore the seed coat colour is determined by the maternal genotype, markers would speed up the selection for slow-darkening (SD) phenotypes as we would not have to subject all breeding lines to storage prior to selection. Markers will greatly facilitate backcrossing of the SD trait into elite pinto lines and introduction of the trait into new crosses. Understanding the inheritance of this trait is only the first step in understanding the cause of seed coat darkening. Identifying the compound(s) responsible for darkening would better enable us to determine the gene(s) involved in this phenomenon. Ultimately, developing a better understanding of this trait would lead to the breeding of pinto bean lines that are resistant to darkening. We could then transfer this trait to other market classes, such as carioca, that also exhibit colour deterioration during storage, shipping and commercial display in polybags.

3.1.2. Staining of pinto bean.

Concern had been expressed regarding yellow staining of seed coats in the pinto crop in 2002, particularly in CDC Pintium, an early maturing cultivar suitable for direct harvest systems. In 2002, about 5% of the harvested beans were affected, enough to cause a price discount for the crop. The cause of this yellow staining was not clear. It was suggested that it could be caused by a pathogen, have genetic origins, or be a one-year environmental interaction. CDC Pintium has been used extensively in western Canadian bean breeding programs, as it is very well

adapted to our growing conditions. It was imperative that we determine the cause of this yellowing before it ruined the reputation of an important new variety and, if the cause was genetic, before it found its way into the next generation of pinto cultivars.

3.1.3. Cooking and canning quality of beans with shiny and matte seed coats.

The seed coat of beans can be shiny or matte. In the navy bean class, which is almost exclusively canned, the preferred types from western Canada have shiny seed coats. The favoured imported black beans in Mexico are the type known as Michigan blacks, which are matte. Apparently, the consumer believes that they have better cooking quality and therefore they will pay more for the matte beans. Anecdotal evidence suggests that the shiny black cultivar Shiny Crow performs well in canning, retaining its colour throughout processing. It is not known, however, if cooking and canning qualities have anything to do with the type of seed coat or are due to some other characteristic of the beans. The importance of tailoring to specific customer needs in pulse markets cannot be overemphasized. For example, Agri-Sales, North America's largest bean exporting company, recently announced that it would not accept delivery of shiny black beans for the Mexican export market. This does not mean, however, that there is not a place for shiny black beans in the canning market. We developed sister lines with shiny and matte seed coats in both navy and black bean types and tested them for canning quality to determine if the presence of a shiny seed coat has any impact.

3.2 Research progress in detail:

3.2.1 Discoloration of pinto beans in storage

3.2.1.1 Accelerated darkening protocol for assessment of seed coat darkening

To determine the genetics of slow-darkening (SD) and to efficiently introgress the trait into new bean cultivars, a quick method to identify slow-darkening individuals would be useful. Chemical analysis of seed coats is destructive, expensive and time consuming. Allowing the seed to naturally darken under room conditions is a lengthy process and could possibly produce variable results from year to year. The objective of this initial study was to develop and validate a non-destructive darkening protocol that can reliably differentiate slow-darkening from regular darkening lines (RD) in a way that is quick, inexpensive, independent of growing and environmental effects, and that will not affect seed germination.

Three different accelerated darkening protocols were compared. The first protocol was conducted in the greenhouse by placing the bean seeds in plastic bags with a 1 cm^2 piece of moistened felt for approx. 2 months. For the second protocol, bean seeds were placed 10 cm below a 254nm UVC lamp for 3 days. For the third protocol, bean seeds were placed in a growth cabinet set at 30°C, 80% relative humidity, and full fluorescent light for several months. All three protocols examined could be used to distinguish darkening beans from slow-darkening beans, however the UVC protocol was considered superior as it was quick, consistent over years, economical and, unlike the greenhouse and the cabinet protocols, had no effect on seed germination.

Fifteen darkening and three slow-darkening genotypes were assessed. Colour measurements using the Hunter L*a*b* classification system were taken at regular intervals during the darkening process. The L*a*b* color system uses three axes to describe color: the L* values run on the z-axis with 100 being perfect white and 0 being perfect black, the a* values run on the x-axis with positive values being more red and negative values being more green, and the

b* values run on the y-axis with positive values being more yellow and negative values being more blue. The changes in the L* and a* values were linear over time and differed between the RD and SD phenotypes. The b* value was erratic over time and was not different between the two phenotypes. Figures 1 and 2, respectively, show the L* and a*color value response over time based on the darkening and slow-darkening lines being pooled together.

Darkening and slow-darkening genotypes were grown indoors in the phytotron and the greenhouse and in the field in multiple locations and the UVC protocol could be used consistently to identify the SD from the RD genotypes. This demonstrated the usefulness of this protocol for screening under any growing conditions.

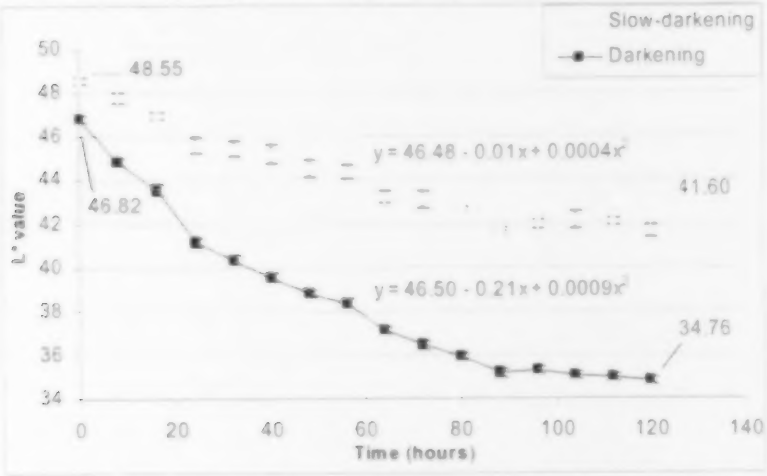

Figure 1. The L* color value response (mean ± SE) of 15 darkening and three slow-darkening pinto bean lines during exposure to UVC light. LSD values: intercept = 7.88, slope = 0.06, quadratic coefficient = 0.0003, end point = 7.80.

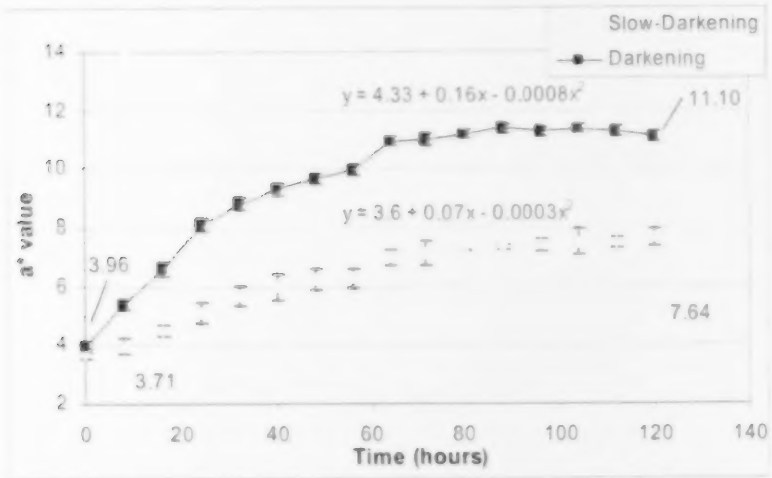

Figure 2. The a* color value response (mean ± SE) of 15 darkening and three slow-darkening pinto bean lines during exposure to UVC light. LSD values: intercept = 0.59, slope = 0.06, quadratic coefficient = 0.0004, end point = 2.60.

This work has been published in Crop Science (Junk-Knievel et al. 2007a).

3.2.1.2 Genetic control of the slow-darkening trait

The inheritance of the SD trait was studied in progeny of crosses between the SD pinto lines 1533-15 and Pinto Saltillo and RD pinto lines, CDC Pintium and HR99.

Methods & Materials

Two F_2 populations were developed from the cross CDC Pintium x 1533-15. These populations were advanced through single seed descent then pooled to develop a single population of 105 $F_{5:6}$ RILs. A population of F_2 plants and 64 $F_{5:6}$ RILs were also developed from the cross HR99 x 1533-15. F_2 plants of all three populations plus the parents were grown in the field at Saskatoon, SK in 2003. Single rows of each RIL from all three populations plus the parents were grown in the field at Saskatoon, SK in 2006. Two additional F_2 populations from the cross CDC Pintium x 1533-15 and its reciprocal were also grown in the field in Saskatoon in 2004 (Table 1).

Table 1 Segregation for seed coat darkening phenotype in F_2 populations derived from crosses among regular darkening (CDC Pintium & HR99) and slow darkening (1533-15 & Pinto Saltillo) lines.

Cross	Environment & year	Regular Darkening	Slow Darkening	$\chi 2_{3:1}$	p-value
CDC Pintium x 1533-15	field 2003	74	26	0.05	0.81
CDC Pintium x 1533-15	field 2003	45	15	0.00	1.00
CDC Pintium x 1533-15	field 2004	70	22	0.06	0.81
1533-15 x CDC Pintium	field 2004	55	11	2.44	0.12
Combined		244	74	0.51	0.48
HR99 x 1533-15	field 2003	67	19	0.39	0.53
Pinto Saltillo x CDC Pintium	field 2004	13	4	0.27	0.60
CDC Pintium x Pinto Saltillo	field 2004	9	2	0.02	0.89
Combined		22	6	0.19	0.66
Pinto Saltillo x 1533-15	field 2004	0	18	-	-
Pinto Saltillo x 1533-15	greenhouse 2005	0	57	-	-
1533-15 x Pinto Saltillo	greenhouse 2005	0	56	-	-

Pinto Saltillo is a photoperiod sensitive, SD pinto cultivar from Mexico (Sanchez-Valdez et al., 2004). F_2 populations were developed from the cross Pinto Saltillo x CDC Pintium, and Pinto Saltillo x 1533-15 to study the allelism of the two SD genotypes. F_2 populations of CDC Pintium x Pinto Saltillo, Pinto Saltillo x CDC Pintium, Pinto Saltillo x 1533-15, and the parents were grown in the field in 2004 at Saskatoon. Due to the day length sensitivity of Pinto Saltillo and some of the F_2 progeny, two additional F_2 populations from 1533-15 x Pinto Saltillo and its reciprocal were developed and grown, along with F_1 and parent plants, in the Agriculture

Greenhouses at the University of Saskatchewan in 2005. Plants were grown on shade benches to restrict the day length to 12 h to ensure flowering of the photoperiod sensitive plants.

Initially it is very difficult to distinguish RD from SD phenotypes. Using the UVC darkening protocol, samples of 15-20 seeds of each parent and F_2 plant or RIL were artificially darkened for 72 hours. Each seed sample was then visually classified as darkening or slow-darkening based on the extent of darkening relative to the unexposed half of the seed coat. In addition, the seed coat colour was quantified following UVC treatment by recording L* and a* colour values of the exposed side of the seeds using a Hunter Lab colorimeter. Chi-square tests were conducted to determine the genetic control of the slow-darkening trait.

Results & Discussion

When classified as RD or SD based on whether or not the seed coat darkened following exposure to UVC light, all F_1 seed coats from crosses involving SD and RD parents were RD. The seed from plants of the F_2 populations derived from crosses between the SD parent, 1533-15, and the RD parents, CDC Pintium or HR99, segregated 3 RD to 1 SD for all crosses (Table 1). Of the 97 $F_{5:6}$ RILs from the cross CDC Pintium x 1533-15 that were homozygous for the darkening trait, 55 were RD and 42 were SD ($\chi2_{1:1} = 1.74$, $p = 0.19$). Homozygous lines from the HR99 x 1533-15 RIL population segregated 30 RD : 33 SD ($\chi2_{1:1} = 0.14$, $p = 0.71$). L* and a* values were bi-modally distributed with a clear separation between RD and SD individuals for both F_2 and RIL populations (Figs.3 and 4). These results clearly indicated single gene control of the darkening phenotype with SD being controlled by a recessive allele.

There was variability within both RD and SD phenotypes for L* and a* values (Figs. 3 and 4) suggesting further influences on the level of darkening within the two phenotypes. One possibility may be variability in maturity resulting in variability in the extent of darkening prior to harvest. Alternatively, there may be modifying genes that influence the full extent of darkening among both the RD and SD phenotypes.

Because Pinto Saltillo is photoperiod sensitive, it begins flowering just prior to the first killing frost at Saskatoon. Progeny from crosses with this parent segregate for photoperiod sensitivity and therefore the number of F_2 plants that set seed is reduced. The seed from F_1 plants of crosses between CDC Pintium and Pinto Saltillo, grown indoors under short days, had the RD phenotype. Field-grown F_2 plants, pooled from two reciprocal crosses and that managed to set seed, segregated 22 RD : 6 SD which is not significantly different from a 3:1 ($\chi2 = 0.19$, $p = 0.89$; Table 1) suggesting single gene control. Similarly, not all F_2 plants of the crosses between 1533-15 and Pinto Saltillo grown in the field reached maturity. Of the 18 that did set seed, all were SD. All plants from the F_2 populations grown in the greenhouse under short days also displayed the SD phenotype suggesting the same gene controlled the SD trait in 1533-15 and Pinto Saltillo and that day length sensitivity did not influence the darkening phenotype in the field. The L* and a* values for the seed coats from the F_2 plants of a 1533-15 x Pinto Saltillo cross grown under short days both followed a normal distribution (Fig. 5) suggesting some quantitative or environmental influence on the results.

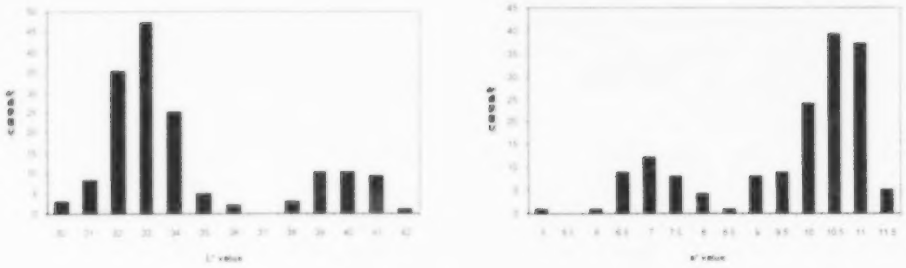

Figure 3. Frequency distribution of L* value (left) and a* value (right) of seed of F_2 individuals from the cross CDC Pintium x 1533-15 and its reciprocal cross grown and harvested from the field in Saskatoon in 2004. Parental colour values: CDC Pintium L* = 32 and a* = 11; 1533-15 L* = 39 and a* = 7.5.

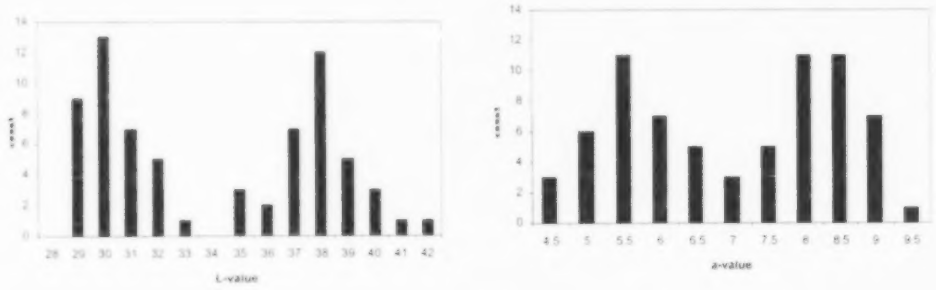

Figure 4. Frequency distributions of L* value (left) and a* value (right) of seed of RILs from the cross CDC Pintium x 1533-15 grown and harvested from the field in Saskatoon in 2006. Parental colour values: CDC Pintium L* = 28, a* = 6.5; 1533-15 L* = 37, a* = 4.

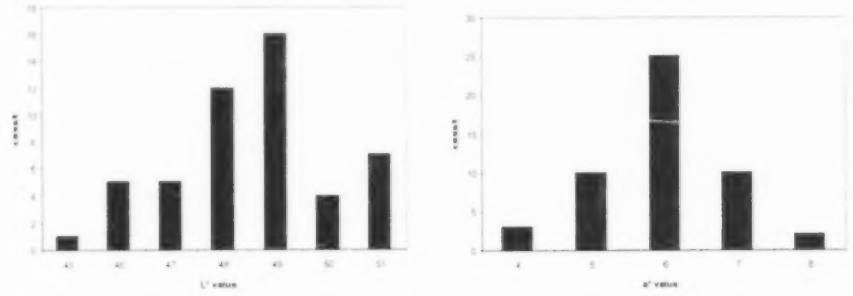

Figure 5. Frequency distribution of L*-value (left) and a*-value (right) of seed of F_2 individuals from the cross 1533-15 x Pinto Saltillo grown and harvested in the greenhouse under short days in 2005. Parental colour values: 1533-15 L* = 48 and a* = 6; Pinto Saltillo L* = 44 and a* = 8. F_1 colour values: L* = 49 and a* = 6. For reference purposes, CDC Pintium L* = 40 and a* = 12.

This work has recently been accepted for publication in Crop Science (Junk-Knievel et al. 2007b).

3.2.1.3 Metabolic profiling of darkening and slow-darkening pinto seed coats

Phytochemical analyses of normal and slow-darkening pinto seed coats were performed to try to identify compounds related to the darkening phenomenon. Flavonoids and tannins were isolated and identified from aged and non-aged seed coats of two pinto bean lines: 1533-15 and CDC Pintium. Aged and non-aged seed coats of both lines were found to contain one main flavonol monomer, kaempferol, and three minor flavonols, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-glucose-xylose, and acetyl kaempferol 3-*O*-glucoside. These compounds were quantified using HPLC-DAD. The combined concentrations of all the kaempferols in seed coats of CDC Pintium were significantly higher than in seed coats of 1533-15, and the combined contents did not change after aging (Fig. 6a). The content of kaempferol decreased nearly by half in the seed coats of CDC Pintium after aging, whereas no significant change was observed in the seed coats of 1533-15. CDC Pintium seed coats, both aged and non-aged, contained significantly more total flavonols than did either aged or non-aged 1533-15 seed coats.

Analysis of the overall level of condensed tannins (CT) using a vanillin assay demonstrated that aged and non-aged seed coats of CDC Pintium had significantly higher levels of CT than did aged and non-aged 1533-15 seed coats (Fig. 6b). CT fractions from both lines, aged and non-aged, were subjected to LC-MS/MS analysis and found to be composed of procyanidins. Procyanidins in the seed coats were predominantly polymers with degrees of polymerization higher than ten. The proportion of these polymers decreased after aging, while the proportion of low-molecular-weight procyanidins increased.

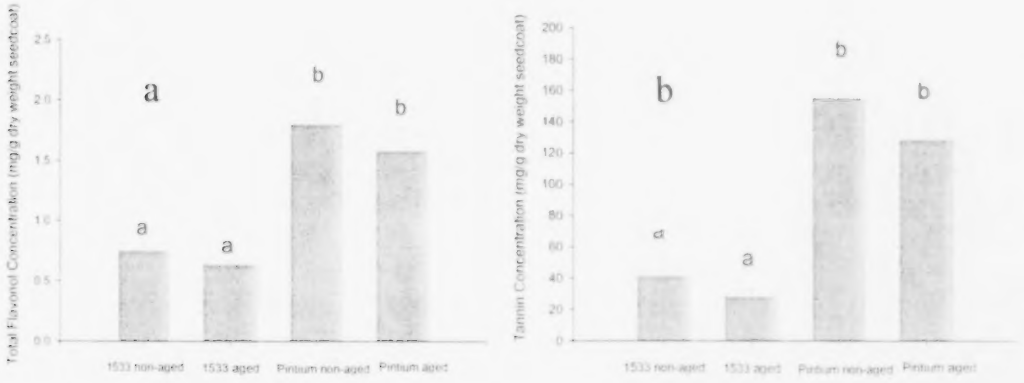

Fig. 6. Total concentration of a) flavanol and b) condensed tannin (as measured using the vanillin test), in aged and non-aged seed coats of 1533-15 and CDC Pintium.

Kaempferol-catechin dimers appear to form in the seed coat and the proportion of these dimers increases as the seed coat ages. This work has been published in The Journal of Agricultural and Food Chemistry (Beninger et al. 2005).

Analysis of the levels of the compounds identified in this preliminary study in the progeny of a cross between CDC Pintium and 1533-15 should allow for the association of specific compounds with the non-darkening trait and give a clearer indication of the biochemical control of this trait. A subset of 7 SD and 10 RD RILs from the CDC Pintium x 1533-15 population used in the genetics study were analysed for kaempferol and CT concentration. The RD lines had significantly higher levels of kaempferol than the SD lines ($p=0.0003$), suggesting the association between the SD trait and decreased levels of this flavonol does exist.

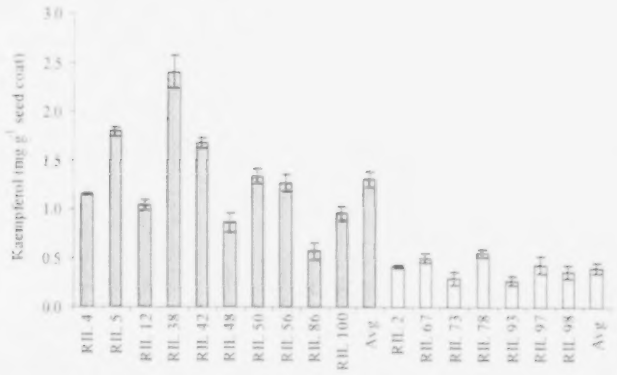

Figure 7: Concentration of kaempferol (aglycone, mg g^{-1} seed coat) in matured seed coat of pinto bean RILs grown in the field. Error bars indicate standard error of the means (n=3). Filled bars, rapid-darkening lines; open bars, slow-darkening lines.

It turns out that analysis of CT levels using vanillin or vanillin analogues is confounded by the presence of other polyphenols, including kaempferol. Analysis using the BuOH-HCl colorimetric method on mature seeds suggests that there is no significant difference in CT content between the SD and RD phenotypes (Fig. 8) although there was a large difference between 1533-15 and CDC Pintium. When mature seeds of the 17 RILs were analysed by HPLC, there was little unbound CT in either group. Most was likely bound in an unextractable form. In comparing the seed coat extracts of the two pinto bean populations during development (sampled at 13 and 20 days after flowering) and at maturity, it was evident that the extractable CT significantly decreased during the time between the 20 DAF samples and matured, harvested seed, over 2 weeks later. Further refinement of the sampling and analytical techniques to assay this bound fraction will be necessary to determine if CT is involved in darkening.

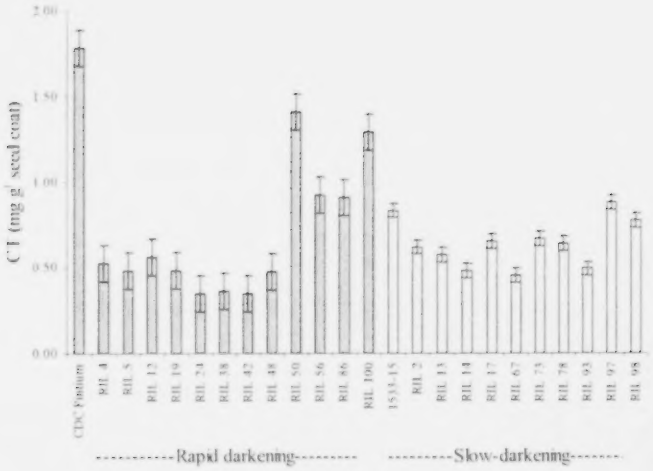

Figure 8: Concentration of condensed tannin in mature seed coat of pinto bean (field-grown, Saskatoon, SK, 2005) quantified by the BuOH-HCl colormetric method. Error bars are standard error of the means. Filled bars, rapid-darkening lines; open bars, slow-darkening lines.

3.2.1.4 Development of a marker for the slow-darkening trait

Zero tannin lentils have bright green stems, while higher tannin lines have a reddish tinge to the stem. We observed that CDC Pintium (RD pinto) has a reddish tinge to its stems while 1533-15 (SD pinto) does not. Populations segregating for seed coat darkening were assessed to determine if the stem colour was correlated with the seed coat darkening phenotype.

Materials & Methods

Individuals from the RIL populations developed above and the parents were grown in a growth cabinet set at 23/18ºC (day/night) with a 12 hour-daylength. Colour assessments were done at the first trifoliate stage. A total of three replicates were grown at different times. Each plant was scored visually for stem colour and classified as 'red' if it had even a tinge of colour or 'green' if it was bright green with no other colour. The first replicate was assessed by one person and the other two by a different person. Following visual assessment, a 1-1.5cm segment of stem was excised from the base of the plant. Reflectance readings were taken using a fibre optic reflectance probe and a diode array spectrophotometer (Ocean Optics Inc., Dunedin FL). The apparatus consisted of a light source, a branched fibre optic reflectance probe, a stand and a test tube clamp to hold the probe, a retractable stage covered in white Teflon® tape and the spectrometer. The Teflon® tape was considered pure white and was used to prevent interference in the reading from the stage. A plastic nose cone was firmly attached to the end of the tube to ensure the distance between the light and the specimen remained the same for each reading. The spectrophotometer was standardized twice daily using dark and white references.

Results & Discussion

Reflectance spectra for Pintium and 1533-15 can be seen in Fig. 9. Note the distinct difference in the region around 540 nm (typical for anthocyanins). RILs were scored visually for seed coat phenotype (RD or SD following artificial ageing). Seedling stem colour assessments

and reflectance measurements were taken from the same stem from plants grown from these seeds. Every time the seeds were SD, the stem was green whether assessed visually or with the aid of the reflectance score (Table 2). The darkening phenotypes were a bit less predictable, but for the most part if the stem appeared to be red based on visual observation the seeds were of the darkening phenotype. Only 5 of the 78 lines rated had green stems but darkening seeds. This suggests that stem colour is a suitable marker for selecting the slow-darkening phenotypes in the seedling stage. This would be of tremendous help in the breeding program where we are backcrossing this trait into various pinto backgrounds and would like to know which F1 has the trait. Surprisingly, the reflectance data were not as predictive as the visual score, with several plants being predicted as green when visually they were in fact red. This could be due to the fact that the pigment is not uniform throughout the stem. A person integrates the colour from the whole stem while the probe is restricted to the small area at which it is pointed, thus there is room for error in the reading. It is not entirely without utility however as it is always able to pick the slow-darkening lines, and includes only a few extra darkening ones.

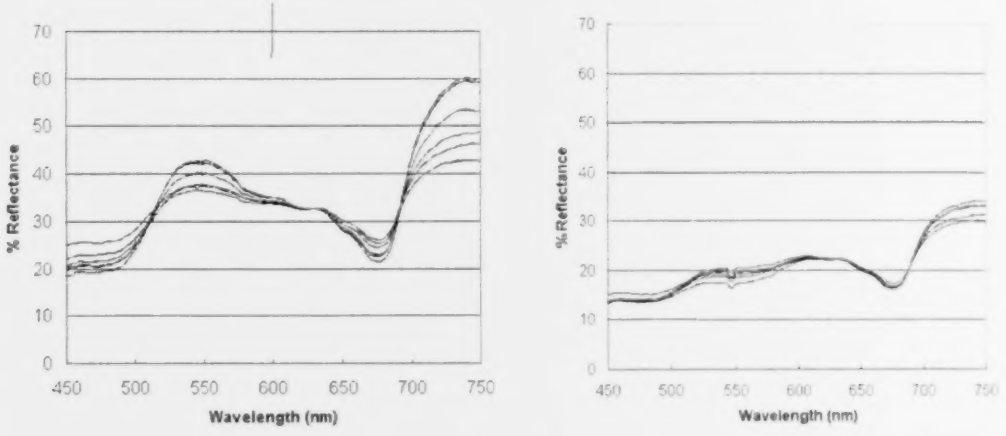

Figure 9: Selected reflectance spectra of hypocotyl segments from CDC Pintium (left) and 1533-15 (right)

Table 2. Comparison of phenotypes based on seed coat darkening (SD = slow darkening; RD = normal pinto), visual assessment of stem colour (red or green) and reflectance score for stem colour (predicted to be red or green).

Seed phenotype	Stem colour (visual)	Reflectance score	Count
SD	green	green	35
RD	red	Red	19
RD	red	green	19
RD	green	green	5

Use of stem colour as a marker for slow darkening is suitable for the breeding program where we can afford to err a little and include some darkening lines in our nursery or crossing program. They will be rogued out in later generations. For genetic analysis and gene identification, however, this is not a suitable marker. Molecular markers are not affected by the environment and are much less subjective than visual ratings. As a result, we are continuing to try to identify molecular markers linked to the gene controlling darkening.

A total of 281 published bean microsatellite and SCAR primers were screened on DNA from the parents of the RILs. Twenty three were polymorphic between the parents so were screened on DNA bulked from ten RD or ten SD individuals. Only five were polymorphic between the bulks and these were subsequently screened on all individuals from the three segregating RIL populations. Only one SSR marker, BM210, was found to be loosely linked to the trait (21cM away). We are currently trying other marker techniques to find better marker(s).

3.2.1.5 Transfer of the slow-darkening trait to other market classes

Since the SD trait is controlled by only one recessive gene, breeding for this trait in pintos is straightforward. The trait can also be transferred to genetically related market classes such as carioca. Carioca beans have the same cream-coloured background as pinto beans and a single gene controls the striped vs spotted pattern phenotype that distinguishes these two classes (Lamprecht, 1947; Bassett, 2007). We seem to have transferred the slow-darkening phenotype to the carioca market class but the seed size is too large so we are continuing to make crosses to improve this seed type. Promising SD individuals have been and will continue to be used as parents in subsequent crosses as well as advanced through the breeding program.

Flor de Mayo (FDM) is another market class with a cream-coloured background and a tendency to after-darken. The pink splash pattern of FDM is also allelic with the stripe and spots of the cariocas and pintos (Bassett, 2007). Interestingly, crosses between 1533-15 and several different FDM lines have always resulted in F_1 seed coats with grey rather than pink pattern colours, and all SD FDM phenotypes that arise in the F_2 have the grey pattern. It would appear that the gene controlling darkening is somehow interfering with the pattern colour.

3.2.2 Yellow staining in pinto beans

The yellow staining does not appear to affect all seeds in a given crop, but the seeds that it does affect are stained all over the seed coat. As we do not know what causes this phenomenon, it is impossible to reproduce to study directly. It also does not occur every year, even in the susceptible varieties.

One suggestion was that the seed staining was the result of infection by bacterial wilt. This disease had recently been identified in beans in Alberta by Dr H. Huang. The results were inconclusive. Bacterial colonies were isolated from the samples but inoculation of susceptible bean plants with the isolated bacteria did not produce any symptoms of bacterial wilt. Additional stained as well as unstained samples were sent for repeat testing. This time, no bacterial colonies were isolated that could be identified as the bacterial wilt pathogen. There were only a few reports of stained Pintium seeds in 2003 and the bacterial wilt pathogen could not be isolated from all samples. It was also noted that many of the seed lots with stained seed came from fields showing no symptoms of the disease. The staining is also much more uniform around the seed than one would expect from a disease infection. It was concluded that it is unlikely that bacterial wilt is the cause of the staining.

Another suggestion was that cold temperatures during seed development could lead to seeds of a specific age to go on to develop yellowed seed coats. Plants of CDC Pintium were grown under controlled conditions with temperatures set to mimic those during flowering in the field in 2002. Individual pods were tagged and checked for staining at maturity. No staining was observed on any of the seeds observed.

CDC Pintium is made up of 49 homozygous sublines, and any one or more could potentially be more predisposed than the others to yellowing. Alternatively, the reason only a subset of the crop yellows is a result of the environment, spatially and or temporally, in which the seed was formed. A genetic fingerprint was generated for each of the 49 sublines and these were compared to the fingerprints for DNA extracted from plants derived from seed that was yellowed. Fingerprinting was done using Amplified Fragment Length Polymorphism (AFLP) analysis. Of the 49 sublines, five generated fingerprints that were indicative of problems with the DNA, resulting in partial digestion of the DNA and, therefore, incomplete banding patterns. These lines were removed from the data analysis. Of the 44 remaining sublines, 33 (75%) were more than 80% similar in their fingerprint and all 44 were more than 70% similar (Fig. 10). This is to be expected from lines that have been selected to be phenotypically similar in the field.

The two lines that represent the plants from yellowed seed clustered with a group of ten sublines and were only 87% similar. This suggests that the yellowed seed did not come from any particular subline. This suggests that no particular subline is more prone to yellowing than another, lending support to the environmental interaction hypothesis.

In the several years since this staining was first observed, several other pinto varieties have been identified that have a similar problem in isolated fields in specific years. The general consensus of breeders and agronomists at a recent bean workers meeting was that it shows up in years with wet harvest conditions. A suggestion to breeders is that they should pay attention to trials that experience wet conditions at harvest as an opportunity to select against lines that are predisposed to yellowing.

Figure 10: Dendrogram showing genetic relatedness of 44 sublines of CDC Pintium and two samples derived from yellow seeded Pintium (Yellow 1 and Yellow 2) based on AFLP fingerprinting.

3.2.3 Cooking and canning quality of beans with shiny and matte seed coats

Sister lines from three different crosses designed to segregate for shiny and matte seed coats were developed and compared for cooking and canning quality. The hypothesis was that the shiny lines would be inferior in quality based on negative comments from the trade and consumers.

Materials & Methods

Crosses were made between AC Black Diamond (shiny black) and CDC Whitecap (matte navy), Envoy (shiny navy) and CDC Expresso (matte black), AC Black Diamond (shiny black) and CDC Expresso (matte black), and Whitecap (matte navy) and Envoy (shiny navy). F_2 populations from each cross were grown in the field in 2003 and problems with germination restricted the number of individuals that survived to maturity. Fifty seeds from each population were selfed using single seed descent in the greenhouse and the field to produce F_5 individuals. Continued problems with germination further reduced the number of individuals in some populations. Seed of 200 F_5 RILs from three populations segregating for shiny or matte and navy or black seed coats were grown in the field in 2005 and bulked for replicated testing in 2006.

In 2006, 75 black-seeded lines that were homozygous for shiny or matte seed coat, plus 5 checks (the parents plus T39) were grown in a 2 rep test at 3 locations (Lethbridge, Preston and SPG). A second test consisting of 19 white-seeded lines plus the same 5 checks was also grown in a 2 rep test at Preston and SPG. Due to seed quantity problems, some lines were not grown at all locations or not in all reps. Following harvest, a total of 40 black-seeded lines, 20 shiny and 20 matte, were selected based on having sufficient seed from each rep at all three locations. An additional 16 white-seeded lines (8 each of shiny & matte) were also selected from the Preston location. These lines plus the checks were canned using standard canning procedures (Balasubramanian et al 2000). Briefly, approximately 150 g of cleaned seed was soaked in dH2O for 16 h, samples were then blanched in dH2O in a steam heated pot for 30 min after reaching 88ºC and cooled to stop the cooking process. Blanched beans were poured into a can, covered with a 1.7% salt solution and sealed. Samples were pressure cooked at 240ºC for 45 min, then stored upside down for two weeks prior to quality assessment.

The following quality parameters were assessed:

1. Hydration coefficient (HC): (wt of soaked beans – can wt)/wt of dry beans (done between blanching and canning.
2. Appearance: 1-5 scale
3. Clumping: 1-3 scale
4. Percent washed drained weight (PWDWT): (washed drained weight/wt of can contents) x 100
5. Texture: in kg/100g sample based on a measurement using a standard shear compression cell of a Kramer shear press.
6. colour measurements using a Hunter Lab colorimeter: L- value measures lightness on a scale of 1-100; a-value measures red-green on a +/- scale; and b-value measures yellow - blue on a +/- scale.

Results & Discussion:

F2 plants from the shiny x matte crosses segregated 3:1 for shiny seed coats, confirming single gene control of seed coat lustre.

Hydration coefficient (HC) is considered to be highly correlated with cooking time (Youssef et al. 1982. Journal of Food Science 47:1695–1697) so a good measure of cooking quality. Analysis of the lines tested in this trial indicated no significant difference in HC between the shiny and matte groups ($p = 0.67$) across all locations and reps. This would suggest that the seed coat lustre does not impact upon cooking time in this set of lines.

There was no significant difference between the shiny and matte groups for PWDWT ($p = .23$). There was no significant difference between the groups for texture and colour at Preston and Lethbridge. There was a significant difference in texture at SPG ($p < 0.0001$) with shiny seeds having, on average, a higher, and therefore better, texture score. Colour values were only assessed for the black lines as mixing in white lines makes the average colour readings meaningless. The shiny back group were on average slightly less "red" and less "yellow" ($a^* = 6.17$; $b^* = 4.38$) than the matte black group ($a^* = 6.55$, $b^* = 5.35$) at SPG. There was a significant difference in the darkness of the two groups ($p = 0.046$) as measured by the L-value (shiny = 21.78, matte = 23.06).

Taken together, it would appear that there is not a large difference between the two groups for cooking or canning quality based on the results from these lines at the three locations tested in 2006. The experiment will have to be repeated in 2007 to confirm these results.

4. Presentations and Publications:

Junk-Knievel, Donna C., Albert Vandenberg, and Kirstin E. Bett. 2007b. Slow Darkening in Pinto Bean (*Phaseolus vulgaris* L.) Seed Coats is Controlled by a Single Major Gene. Accepted for publication in Crop Sci., June 2007.

Junk-Knievel, Donna C., Albert Vandenberg, and Kirstin E. Bett. 2007a. An Accelerated Postharvest Seed-Coat Darkening Protocol for Pinto Beans Grown across Different Environments. Crop Science 47:692–700.

Bett, K., Junk, D. and Vandenberg, B. 2006. Slow Darkening Pinto Beans. Oral presentation at the 6th Canadian Pulse Researchers Workshop. Saskatoon SK Nov 1-3, 2006.

Marles, M.A. Susan, Vandenberg, Albert, and Bett, Kirstin E (2006). Elucidation of the factors that contribute to the after-darkening phenomenon in pinto bean. 23rd International Conference on Polyphenols. August 21-25, Winnipeg, Manitoba, Canada. pp 431-432. (poster abstract)

Bett, K., Junk, D. and Vandenberg, B. 2005. Slow Darkening Pinto Beans. Oral presentation at the Bean Improvement Co-operative Biennial Meeting Newark Delaware Nov. 2005.

Junk, D. C. 2005. Seedcoat darkening in pinto beans. MSc Thesis. University of Saskatchewan.

Beninger, C.W., Gu, L., Prior, R.L., Junk, D.C., Vandenberg, A. and Bett, K.E. 2005. Changes in Polyphenols of the Seed Coat During the After-Darkening Process in Pinto Beans (*Phaseolus vulgaris* L.) J. Agr. Food Chem. 53:7777-7782.

Donna Junk, Bert Vandenberg and Kirstin Bett. 2005. Post harvest seed coat darkening in pinto beans (*Phaseolus vulgaris*). Poster and abstract. Proceedings of the International Edible Legume Conference Durban, S. Africa 17 - 21 April 2005

Junk, D., Bett, K. and Vandenberg, A. (2005) The Effect of Variety and Environment on the Colour of Pinto Beans. Poster at Pulse Days 2005.

Junk, D., Vandenberg, A. and Bett, K. Screening and genetic control of slow-darkening pinto beans. Oral presentation at the 5th Canadian Pulse Workers Workshop, London ON, Nov. 2004

Junk, D., Vandenberg, A. and Bett, K. (2004) When is a pinto a pinto? Ann. Rep. Bean Improv. Co-op. 47: 141-142

Junk, D., Bett, K. and Vandenberg, A. (2004) Maintaining colour and price of pinto beans. Poster at Pulse Days 2004 and Soils and Crops 2004.

5. Information of benefit to producers, processors, or governments:

5.1 New slow-darkening beans

The slow darkening pinto line 1533-15 is now in seed and commercial production and was tendered by the Saskatchewan Pulse Growers to Walker Seeds, a Saskatchewan-based company. They will have exclusive access to future breeding lines of SD for a defined number of years, allowing them to invest in the necessary marketing to develop a value chain that links growers with processors, exporters and importers based on the enhanced economic value potential of the slow-darkening beans. The slow-darkening pinto beans are generating commercial interest at home and abroad and the first commercial crop is being grown in the summer of 2007. Research initiated on this project has spawned a number of other spin off projects, including a cooking study and more in depth biochemical analyses. Slow-darkening lines of carioca beans, which have similar seed coat genetics to pinto but with a different pattern distribution, are in the final stages of testing and should be released to potential commercial partners in the next 2-3 years. Thus far, we have not seen any negative effects of the slow-darkening trait on bean production or seed quality.

5.2 Management for improved seed quality

The best advice for producers who are growing pinto beans, and any other bean for that matter, would be to make sure that harvest is done in a timely manner. Leaving pinto beans out in the field, especially under wet conditions, can lead to problems in quality such as yellowing of the seed coat. It will also accelerate darkening in RD pinto beans.

5.3 Cooking and canning quality

Based on the limited results so far on the cooking and canning quality of shiny vs matte beans, it would appear that a blanket statement like "shiny beans don't can well" may not hold

up for all genotypes. Further investigation into this matter is warranted if producers want to grow cultivars with shiny seed coats.

5.4 Marketing impact

Much of the research conducted as part of this project will help Saskatchewan bean exporters develop a science-based marketing strategy in domestic and overseas markets. Our understanding of genetic control of the seed coat darkening phenomenon for pinto beans will from the basis for establishing improved economic value relative to regular pinto beans, forming the basis of a value chain for marketing in the highest priced sector of the bean market.

6. Personnel

Field, lab and greenhouse technical support.

7. Equipment purchased or rented:

Lab and field equipment rental.
Growth facilities rental.
No major equipment was purchased for this project.

Acknowledgements:

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